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Intermittent cold exposure causes a muscle-specific shift in the fiber type composition in rats

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We examined the effect of long-term intermittent cold exposure on the fiber type composition of the predominantly type I soleus and the predominantly type IIb extensor digitorum longus (EDL) muscles of rats. Cold exposure was accomplished by submerging the rats in shoulder-deep water, maintained at 20+0.5 degrees C, for 1 h/day, 5 days/wk, for <19 wk. The efficacy of the treatment was tested by subjecting both groups to 20 degrees C water for 45 min while rectal temperature (Tre) and O2 consumption (Vo2) were measured. The cold-exposed group displayed a 22% smaller reduction in Tre ($P<0.05$) at the end of the exposure and 23% greater Vo2 ($P<0.05$) during the same period. The present study demonstrates that intermittent cold exposure induces a type I-to-type IIa transformation in the soleus muscle while having no influence on the EDL muscle.

Acclimation hypothermia mitochondria metabolism

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Intermittent cold exposure causes a muscle-specific shift in the fiber type composition in rats

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WALTERS, T. J., AND S. H. CONSTABLE. *Intermittent cold exposure causes a muscle-specific shift in the fiber type composition in rats.* J. Appl. Physiol. 75(1): 264–267, 1993.—We examined the effect of long-term intermittent cold exposure on the fiber type composition of the predominantly type I soleus and the predominantly type IIb extensor digitorum longus (EDL) muscles of rats. Cold exposure was accomplished by submerging the rats in shoulder-deep water, maintained at 20 ± 0.5°C, for 1 h/day, 5 days/wk, for ≤19 wk. The efficacy of the treatment was tested by subjecting both groups to 20°C water for 45 min while rectal temperature (T_{re}) and O_2 consumption ($\dot{V}O_2$) were measured. The cold-exposed group displayed a 22% smaller reduction in T_{re} ($P < 0.05$) at the end of the exposure and 23% greater $\dot{V}O_2$ ($P < 0.05$) during the same period. Fiber type composition was determined using routine histochemical methods for myosin-adenosinetriphosphatase. In the soleus muscle of the cold-exposed rats, the number of type IIa fibers increased 156% ($P < 0.05$) and the number of type I fibers decreased 24% ($P < 0.05$). Cold exposure had no significant influence on the fiber type composition of the EDL muscle. Cold exposure resulted in an increase in citrate synthase activity of 20 and 22% in the soleus and EDL muscles, respectively ($P < 0.05$). The present study demonstrates that intermittent cold exposure induces a type I-to-type IIa transformation in the soleus muscle while having no influence on the EDL muscle.

acclimation; hypothermia; mitochondria; metabolism

MUSCLE FUNCTION is affected by temperature. As a result of the Q_{10} effect, a decline in muscle temperature results in a reduction in the rate of flux through enzyme systems, which include myosin-adenosinetriphosphatase (M-ATPase) and the mitochondrial enzymes involved in ATP production. A reduction in M-ATPase activity results in a slower speed of muscle contraction, whereas the reduction in mitochondrial enzyme activity results in a reduced rate of ATP production. In poikilotherms, such as many species of fish and lizards, acclimation to seasonal declines in temperature results in an increase in M-ATPase activity (6, 16, 17) as well as an increase in mitochondrial enzyme activity (6, 15). These adaptations partially compensate for the temperature-induced decline in muscle function.

Although increases in skeletal muscle mitochondrial enzyme activity (9, 10) and M-ATPase activity (4) have been observed in cold-acclimated mammals, alterations in fiber type composition have not. There are no reports of fiber type composition in cold-acclimated mammals perhaps because the typical method for inducing cold acclimation in mammals is exposure to cold air (1, 9, 10). Water has a much greater heat capacity than air and, therefore, results in a much greater loss of heat, thus possibly providing a greater stimulus intensity for the induction of a fiber type shift.

We tested the hypothesis that a shift in fiber type composition would occur in rats if they were given an adequate cold stimulus. Rats were exposed daily (1 h) to cold water (20°C) over 17–19 wk. Additionally, to indirectly compare this method of cold exposure with traditional methods involving cold air, skeletal muscle oxidative enzyme activity and whole body metabolic profiles were also examined.

METHODS

Animal care. All animals were procured, maintained, and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources-National Research Council. Twenty male rats (Sprague-Dawley CD-VAF/Plus), initially 353 ± 28 g, were obtained from the colonies of Charles River Laboratories (Wilmington, MA). Rats were multiply housed, four rats to a cage, until they reached >500 g body wt, at which point they were housed two rats per cage. Rats were allowed ad libitum access to food (Purina Rodent Chow) and water. The room in which the rats were kept was maintained on a 12:12-h light-dark cycle at 25 ± 1°C.

Experimental treatment. Rats were randomly assigned to a control (CON) or a cold-exposd (CE) group. Cold exposure was accomplished by submerging the rats in shoulder-deep water in a 50-gal container, with five rats per container. The water temperature was constantly monitored and was maintained at 20.0 ± 0.5°C by the periodic addition of ice cubes. Initially, the rats were exposed for 5 min/day, with an additional 5 min added each day until a final period of 60 min/day was reached. The CE rats received this treatment 5 days/wk for 17–19 wk.

Efficacy of treatment. To evaluate the efficacy of cold exposure, CON ($n = 5$) and CE ($n = 5$) rats were exposed to cold water while rectal temperature (T_{re}) was monitored with a Vitec thermal probe inserted 5 cm into the rectum (Fig. 1). In addition, the O_2 consumption ($\dot{V}O_2$) during cold exposure was determined (Fig. 2). Because of the drastic decline in the T_{re} of the CON rats, the exposure was terminated after 45 min in this group. The exposure involved placing a rat in a cylindrical metabolic chamber (750 cm^3), which was in turn placed in the water, as previously described. The metabolic chamber

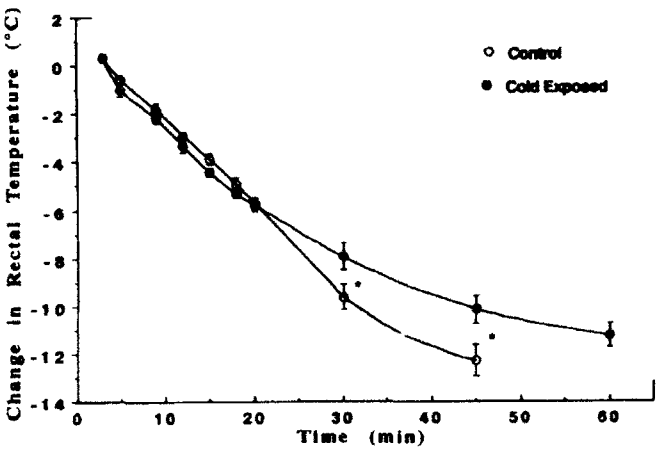

FIG. 1. Influence of cold water immersion (20°C) on rectal temperature in control and cold-exposed rats. Rectal temperature before exposure (i.e., *time 0*) was 37.2 ± 0.1 and 37.4 ± 0.1°C for control and cold-exposed groups, respectively. * Significantly different from control ($P < 0.05$).

had holes up to a point corresponding to 1 cm above the water line. The holes below the water line allowed for the mixing of water between the chamber and the 50-gal container, while the holes above the water line allowed the entry of air, which was pulled through the chamber and exited through a mixing chamber at the top. Exhaust was pulled by at a rate of 600 ml/min, and fractions of expired O_2 and CO_2 were determined in line with a Perkin-Elmer 11000 medical gas analyzer. The $\dot{V}O_2$ was computed by a Macintosh II computer interfaced with the gas analyzer and flowmeter by use of the Lab View data acquisition/analysis software package, as previously described (3). The rats on which these data were determined were familiarized with the procedure by periodic placement in the metabolic chamber and into the water before the actual experiment.

Food intake. The daily food intake of the rats was estimated by determining the amount of food consumed per cage for the last week of the study. This value was divided by 2 (2 rats/cage) and then divided by 7 (7 days/wk).

Histochemical analysis. Rats were killed with an overdose of pentobarbital sodium (Nembutal). The soleus and extensor digitorum longus (EDL) muscles were excised, weighed, and pinned to a wooden stick. The muscles were then frozen in isopentane cooled (−100°C) in liquid N_2. Cryostat sections (8 μm) were stained for M-ATPase by use of routine histochemical methods (8) at pH 4.53 and 4.30. The fiber type of each muscle was determined from three photomicrographs per muscle. This represented 250–350 fibers/photomicrograph. The fibers were classified according to the nomenclature of Staron and Pette (23).

Citrate synthase activity. Citrate synthase activity was determined in muscle homogenates by the spectrophotometric method of Srere (22).

Statistics. Significance between groups was determined using a *t* test. The level of significance was preset at $P = 0.05$.

RESULTS

Animal weights and food intake. There was a significant difference in the body weights of the CE group compared with the CON group: 594 ± 9 vs. 630 ± 10 (SE) g, respectively ($P < 0.05$). However, the CE rats consumed 37% more food ($g \cdot day^{-1} \cdot g$ body wt^{-1}) than the CON rats ($P < 0.05$).

Efficacy of the cold exposure. The severity of the cold exposure in the present study can be seen in Fig. 1. Both CON and CE rats underwent a significant reduction in T_{re} in response to the treatment. However, the rate and the amount of reduction in T_{re} were significantly less in the CE group during the exposure. The ability of the CE group to maintain a higher T_{re} was accompanied by a significantly greater metabolic rate during the exposure

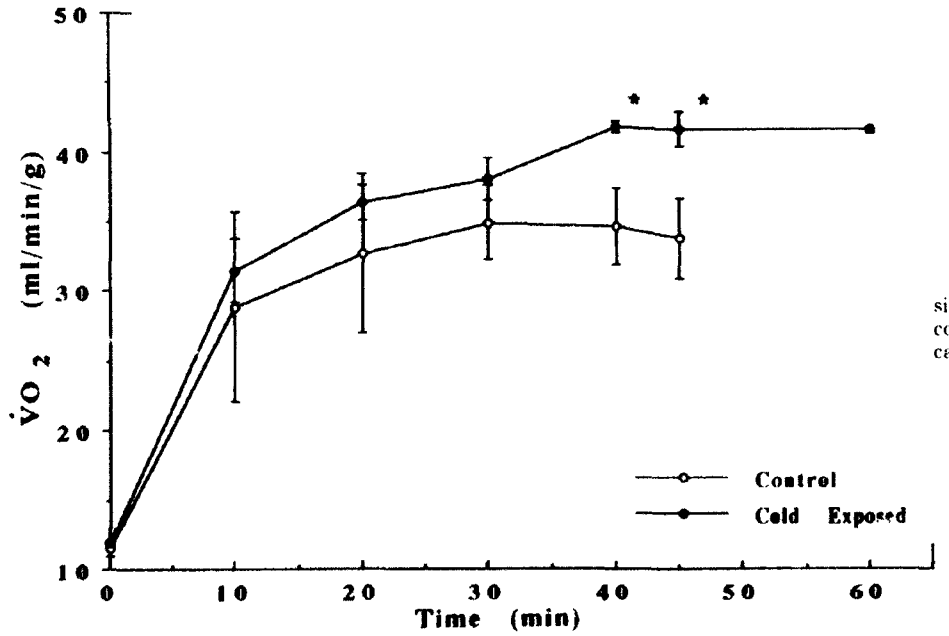

FIG. 2. Influence of cold water immersion (20°C) on O_2 consumption ($\dot{V}O_2$) in control and cold-exposed rats. * Significantly different from control ($P < 0.05$).

TABLE 1. *Fiber type composition of soleus and EDL muscles from CON and CE rats*

Group	Type I	Type Ic	Type IIa	Type IIb
		Soleus		
CON	80.9±3.0	7.0±3.1	12.1±0.3	
CE	64.7±3.9*	4.3±1.6	31.0±3.3*	
		EDL		
CON	2.9±0.6		23.6±0.9	76.4±0.9
CE	3.8±1.0		25.7±6.1	71.0±6.1

Values are means ± SE of 5 rats/group expressed as percentages. CON, control; CE, cold exposed; EDL, extensor digitorum longus. * Significantly different from CON ($P < 0.05$).

(Fig. 2). The relative difference of $\dot{V}O_2$ and T_{re} between the CE and CON rats was 22 and 23%, respectively.

Fiber type composition. Cold exposure had a significant impact on the fiber type composition of the soleus muscle: the number of type IIa fibers in the CE rats increased threefold (12.1 ± 0.3 vs. 31.0 ± 3.3%, $P < 0.05$), and the number of type I fibers decreased 24% (80.9 ± 3.0 vs. 64.7 ± 3.9%, $P < 0.05$; Table 1). Cold exposure had no significant influence on the fiber type composition of the EDL (Table 1).

Citrate synthase activity. Cold exposure resulted in a 22% increase ($P < 0.05$) in citrate synthase activity in the soleus muscle (27.0 ± 1.2 vs. 32.9 ± 0.9 $\mu mol \cdot min^{-1} \cdot g^{-1}$). Citrate synthase activity in the EDL muscle was 23% greater in the CE than in the CON rats ($P < 0.05$, 22.3 ± 0.6 vs. 27.4 ± 1.1 $\mu mol \cdot min^{-1} \cdot g^{-1}$).

DISCUSSION

The cold exposure treatment used in the present study clearly resulted in a number of adaptive responses. The increase in oxidative enzyme activity that occurred in the soleus and EDL muscles of the rats in the present study is consistent with a number of earlier reports for both mammals (9, 10) and poikilotherms (7, 15). Temperature has a profound influence on skeletal muscle: the rate of metabolic flux is reduced as temperature declines. An increase in oxidative enzyme activity thus serves as a positive adaptation to the cold.

The present study demonstrates that sufficient intermittent cold exposure can induce a fiber type shift in predominantly type I fibers in rats. This finding is consistent with reports of decreased M-ATPase activity in muscle homogenates of cold-adapted fish (12, 16) and rats (4). As the temperature of skeletal muscle declines, so does its contractile speed (5, 20, 21). In the soleus muscle, the shift in fiber type from slow-contracting type I fibers to an increasing percentage of faster-contracting type IIa would compensate for the temperature-induced reduction in contractile speed. Consistent with this reasoning is the lack of a shift in the predominantly type IIb EDL muscle; i.e., it is already predominantly composed of the fastest-contracting muscle fibers.

The cold exposure treatment used in the present study would be expected to result in elevated circulating thyroid hormone (TH) levels (1, 13, 18, 19). Rats that are exposed to cold air (−15°C) for 1 h undergo a twofold increase in serum triiodothyronine (18). Under these conditions, rats display a 1.5°C decline in T_{re}. In addition, chronic cold exposure results in an increase in TH clearance rate (19). A much greater increase in TH levels would be expected in the present study, because the rats experienced a 10–12°C decline in T_{re}. A recent study examining the influence of cold exposure on myosin heavy chain (MHC) expression in cardiac muscle demonstrated an increase in the proportion of the δ-MHC with a concomitant increase in circulating TH levels (1). In the soleus muscle, elevated TH levels in rats result in an increase in the expression of the IIa MHC (1) and intermediate and fast myosin isoforms (2) accompanied by a reduction in the slow myosin isoform (2). In contrast, the EDL muscle is not affected by elevated TH levels (1, 2). Finally, TH is a potent stimulus for increased oxidative enzyme activity (24). Taken together, these data provide indirect support for the possible role of TH in mediating the alterations reported in the soleus and EDL muscles after cold exposure in the present study.

Alternatively, Hazel and Prosser (11) proposed that cold exposure alone may induce the expression of temperature-specific isozyme genes. If this is true, cold water may be more effective than cold air in inducing a fiber type shift, because the greater heat capacity of water would be expected to result in a greater reduction in local muscle temperature than would occur with cold air. Furthermore the increase in oxidative enzyme activity reported here is greater than that reported for rats chronically maintained in cold air (5°C) (10).

Cold exposure clearly resulted in adaptations that were manifested in a slower rate of temperature decline in the CE rats (Fig. 1). Although not measured in the present study, the significantly greater $\dot{V}O_2$ displayed by the CE rats in Fig. 2 likely reflects an adaptive increase in the mass and metabolic activity of brown adipose tissue (10). Additionally, the significantly greater food intake in the CE rats represents another important manifestation of cold adaptation (10).

In conclusion, long-term intermittent cold exposure by use of cold water (20°C) for 1 h/day induced a significant type I-to-type IIa shift in the soleus muscle as well as significant increases in oxidative enzyme activity in the soleus and EDL muscles of rats. The metabolic adaptations induced by this treatment mode are comparable to those reported for chronic cold air exposure.

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